EP0474874

Publication Title:

MAILLARD REACTION INHIBITOR.

Abstract:

Abstract of EP0474874

A Maillard reaction inhibitor containing a substance represented by general formula (I), a pharmaceutically acceptable ester thereof, and a pharmaceutically acceptable salt of the substance or the ester, wherein X represents hydroxyl or nitro. It is used for treating or preventing various complications of diabetes, such as coronary artery disease, peripheral circulatory disturbance, cerebrovascular disease, neurosis, nephropathy, arteriosclerosis, arthrosclerosis, cataract and retinitis, and similar diseases caused by aging, such as atherosclerosis, coronary heart disease, cerebrovascular disease, senile cataract, and so forth. Data supplied from the esp@cenet database - Worldwide aae

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 474 874 A1**

(12) EUROPEAN PATENT APPLICATION published in accordance with Art. 158(3) EPC

(21) Application number: **91903819.0**

(22) Date of filing: **08.02.91**

(86) International application number: **PCT/JP91/00200**

(87) International publication number: **WO 91/11997 (22.08.91 91/19)**

(51) Int. Cl.[5]: **A61K 31/195**

(30) Priority: **19.02.90 JP 39241/90**

(43) Date of publication of application: **18.03.92 Bulletin 92/12**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IT LI LU NL SE**

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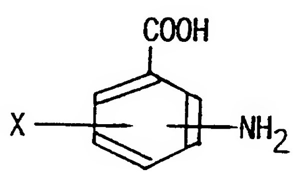
(54) MAILLARD REACTION INHIBITOR.

(57) A Maillard reaction inhibitor containing a substance represented by general formula (I), a pharmaceutically acceptable ester thereof, and a pharmaceutically acceptable salt of the substance or the ester, wherein X represents hydroxyl or nitro. It is used for treating or preventing various complications of diabetes, such as coronary artery disease, peripheral circulatory disturbance, cerebrovascular disease, neurosis, nephropathy, arteriosclerosis, arthrosclerosis, cataract and retinitis, and similar diseases caused by aging, such as atherosclerosis, coronary heart disease, cerebrovascular disease, senile cataract, and so forth.



(I)

EP 0 474 874 A1

Technical Field

This invention relates to the inhibition of denaturation reaction of proteins by reductive sugars such as glucose. which is known by the name of Maillard's reaction. More specifically this invention relates to the inhibition of the formation of Amadori rearrangement products which originate from non-enzymatic bond formation between glucose and proteins.

Background Art

The reaction in which proteins turn brown by reacting non-enzymatically with reductive sugars such as glucose (hereinafter referred to as "the glycosylation") was first reported by Maillard in 1912 [Maillard, L.C., Compt. Rend. Soc. Biol., 72:599 (1912).] Since then, the reaction has been widely recognized by the name of Maillard's reaction in the field of food chemistry. For example, it has been noted that proteins react with glucose in stored or heated food, generate a brown color and finally are denaturated by formation of cross-linkings among molecules.

Later, attention was directed to reactions of glucose with proteins which may occur in living bodies when Rahbar reported that the level of Hb_{A1c}, a minor component of hemoglobin, was found elevated in red blood cells of diabetic patients [Rahbar, S., Clin. Chim. Acta, 22:296 (1968).] And, through structural analysis of Hb_{A1c}, it has been confirmed that Maillard's reaction occurs in living bodies.

The mechanism of Maillard's reaction in living bodies has been presented by Brownlee et al. [Brownlee, M. et al., Science, 232:1629 (1986).] The reaction proceeds as follows.

At first, the aldehyde group of the open-ring structure of glucose reacts with an amino group in protein molecule to form a schiff's base. The resulting schiff's base is unstable and is rapidly converted non-enzymatically into Amadori rearrangement product via intra-molecular rearrangement reaction. If this protein is maintained for a long period of time within the body, the rearranged product undergoes a gradual dehydration reaction to form a new glucose derivative. This derivative then irreversively forms cross-linkings with a variety of molecules including proteins to form bridges among molecules, thus yielding aggregation products of, chiefly, proteins.

This type of product resulting from advanced reactions of glycosylated proteins is usually abbreviated to AGE (Advanced Glycosylation End product.)

In parallel to the formation of AGE, biological adaptibility of the protein is lowered, and the protein becomes less soluble and more resistant to proteases and, in many cases, turns yellow-brown and becomes fluorescent.

Though also observed in healthy human, Maillard's reaction is markedly noted in those with diabetes mellitus, which is characterized by the elevation of blood glucose. Maillard's reaction is especially notable in proteins with a slower rate of metabolic turnover, for example crystallins, which are the structural proteins in the lens, and collagens. While a variety of disorders, for example neuropathy, cataract, nephropathy, retinopathy, arthrosclerosis and atherosclerosis, are noted as complications of diabetes mellitus, these disorders bear a very close resemblance with disorders noted quite frequently in the aged human.

It, therefore, is regarded that AGE is also formed gradually from proteins with a slower turnover rate by glycosylation with glucose even at a nomal level of blood sugar.

With this background, efforts have been made to find compounds which may inhibit Maillard's reaction within living bodies. An example of such efforts has been shown by Brownlee as cited who reported that aminoguanidine inhibits Maillard's reaction in vitro and suppresses AGE formation in arterial walls of diabetic rats in vivo. In Japanese Patent Publication Kokai No. 142114/87, it has been suggested that aminoguanidine, α-hydrazinohistidine and lysine may block the active carbonyl group of Amadori rearrangement products to inhibit AGE formation. It has also been disclosed that different compounds may suppress Maillard's reaction. Such compounds include thiosemicarbazides, 1,3-diaminoguanidine and benzoylhydrazine (Japanese Patent Publication Kokai No. 56614/89), and various derivatives of guanidine (Japanese Patent Publication Kokai No. 83059/89.)

In the patent publications cited above, researches for inhibitors of Maillard's reaction were made using the amount of AGE, the end product of Maillard's reaction, as an index. The present inventor, instead, took the inhibition of formation of Amadori rearrangement product as an index in the investigation. This was based on an estimation that a markedly effective inhibition of Maillard's reaction may be expected by inhibiting the very formation of Amadori rearrangement product, which is the immediate causing factor in protein aggregation process in Maillard's reaction.

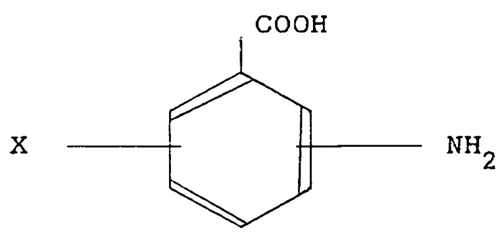
Bruggemann et al. [J. Bruggemann et al., Lebensm. Unters. Forsch., 137:137-143 (1968)] and Finot et al. [P.A. Finot et al., Experientia, 24:1097-1099 (1968)] have reported that the amount of ϵ-N-(furoyl-methyl)-

L-lysine (hereinafter referred to as "furosine"), which is an Amadori rearrangement product resulted from non-enzymatic glycosylation of ε-amino residue of lysine in proteins, may be taken as an index of the non-enzymatic glycosylation of protein molecules. The present inventor made an intensive research for the optimal experimental condition for formation of furosine from protein dissolved in water containing glucose, and, according to the condition thus established, evaluated various compounds for the presence and strength of inhibitory effect on furosine formation.

As a result, the present inventor discovered that some of the derivatives of aminobenzoic acids have a potent inhibitory effect on furosine formation. Then, evaluation was continued, which lead to the accomplishment of the present invention.

Disclosure of Invention

Thus the present invention is a pharmaceutical composition for inhibition of Maillard's reaction characterized in that it contains a compound of the formula (I),



(I)

wherein X denotes a hydroxyl group or a nitro group, a pharmaceutically acceptable ester thereof or a pharmaceutically acceptable salt of the said compound or the said ester.

Examples of the pharmaceutically acceptable esters of the compound (I) include lower alkyl esters of the carboxyl group of the compound such as methyl ester, ethyl ester, n-propyl ester and isopropyl ester, and esters of the phenolic hydroxyl group of the compound such as esters with lower carboxylic acids including acetic acid ester, oxalic acid ester, malonic acid ester, maleic acid ester and succinic acid ester, and esters with inorganic acids including phosphoric acid ester.

Examples of suitable salts of the compound (I) or pharmaceutically acceptable esters thereof include, in particular, alkali metal salts thereof such as sodium salt and potassium salt, alkaline earth metal salts thereof such as calcium salt and magnesium salt, and salts thereof with inorganic acids such as hydrochloric acid, sulfuric acid and phosphoric acid, or with organic acids such as acetic acid and maleic acid.

The scope of the present invention, however, is not limited by these examples, and salts which are usually accepted as pharmaceuticals are included in the scope of the present invention.

The Maillard's reaction inhibitors of the present invention may be used for the treatment or prophylaxis of a variety of disorders mentioned later which may develop via Maillard's reaction. For the purpose, the inhibitors of Maillard's reaction of the present invention may be administered orally or non-orally. For non-oral administration, the inhibitors may be administered parenterally for systemic purpose or topically, for example, in the form of eye drops.

The Maillard's reaction inhibitor of the present invention may be administered orally at a dose - as the compound (I) - of, generally, 1 to 1,000 mg/day, more preferably 5 to 200 mg/day. For injection, the dose may be generally 0.1 to 100 mg/day, more preferably 1 to 50 mg/day.

For eye drops, it may be applied in the form of liquid at a concentration of, generally, 0.05 to 5.0 w/v %, more preferably 0.1 to 2.0 w/v %.

However, the examples above are not intended to limit the dose range. A suitable dose may be set according to the type and severity of disorders and schedules of treatment in each case.

The Maillard's reaction inhibitor of the present invention may be formed into, for example, tablets, pilles, powder, granules or capsules for oral administration, aqueous or non-aqueous solution, suspension or emulsion for injection, or eye drops or eye ointment for ophthalmic topical use.

For preparing pharmaceutical composition of the present invention into the form of tablets for oral administration, ingredients usually incorporated in tablet preparation may suitably be utilized.

Such ingredients include, for example, diluent bases such as hydroxypropylcellulose, crystalline cellulose, corn starch, polyvinylpyrrolidone and magnesium metasilicate aluminate, lubricants such as

magnesium stearate, disintegrators such as fibrinous calcium gluconate, and solubilizers such as glutamic acid and aspartic acid.

For preparing a pharmaceutical composition of the present invention in the form of aqueous injection, ingredients usually incorporated in injectable preparations may suitably be utilized. Such ingredients include, for example, buffering agents such as phosphates, preservatives such as chlorobutanol, stabilizers such as sodium sulfite, and isotonizers such as sodium chloride.

For preparing a pharmaceutical composition of the present invention into the form of eye drops, ingredients usually incorporated in the formation of eye drops may suitably utilized. Such ingredients include, for example, buffering agents such as phosphates, borates, acetates and citrates, preservatives such as chlorobutanol, methylparaben, propylparaben, benzalkonium chloride and chlorhexidine digluconate, stabilizers such as sodium sulfite, sodium bisulfite and sodium edetate, isotonizers such as sodium chloride, potassium chloride, mannitol, sorbitol and glycerol, and solubilizers such as polysorbate 80 and cyclodextrins.

(Pharmacological test)

The effect of the Maillard's reaction inhibitors of the present invention was determined as follows using the test compounds listed below.

They are known compounds and were purchased from the market.

AB-1: 5-hydroxyanthranilic acid
AB-2: 3-hydroxyanthranilic acid
AB-3: 4-nitroanthranilic acid
AB-4: 5-aminosalicylic acid
AB-5: 4-aminosalicylic acid
AB-6: 3-aminosalicylic acid
AB-7: 3-amino-4-hydroxybenzoic acid

(Test methods)

Sample solutions as shown below were aseptically prepared from bovine serum albumine (No. A-8022, Sigma)(hereinafter referred to as BSA), 50 mM phosphate buffer solution (pH 7.3) and the test compounds listed in Table 1 and aminoguanidine.

The sample solutions were kept for 4 weeks at 37 °C, and the amount of furosine which was formed by non-enzymatic glycosylation was determined by HPLC according to the method of Schleicher et al. [J. Clin. Biochem., 19:81-87 (1981).] Thus, the sample solutions after reaction were dialyzed, and aliquots of 1 ml were lyophylized and then hydrolyzed by the addition of 1 ml of 6 N hydrochloric acid followed by heating at 100 °C for 20 hours. After removal of hydrochloric acid by evaporation, 1 ml of water was added to each sample, and the samples were subjected to filtration using a filter with the pore size of 0.45 μm. The filtrate was used as the sample for HPLC. ODS-120T (Tosoh Corporation) was used for the column and 7 mM phosphoric acid solution was used as the eluant. The absorbance peak whose ratio of peak area at 280 mm/254 mm was 3.9/1 was regarded as the peak corresponding to furosine.

[Constituents in the phosphate buffer solution]

Normal sample; 20 mg/ml BSA
Control sample; 20 mg/ml BSA and 50 mM glucose
Test sample; 20 mg/ml BSA, 50 mM glucose and 5 mM test compound

Upon the area of the peak of furosine of each sample, the inhibition rate of furosine formation by the test compound was calculated as follows.

$$\text{Inhibition rate (\%)} = (c-d) \div (c-n) \times 100$$

c; peak area of furosine of the control sample
d; peak area of furosine of the test sample
n; peak area of furosine of the normal sample

(Results)

As shown in Table 1, each of the test compounds, AB-1 to AB-7, exhibited a remarkably potent inhibitory effect in comparison with aminoguanidine, a known inhibitor of Maillard's reaction.

Table 1

Test compound	Inhibition rate (%)
AB-1	94.1
AB-2	69.4
AB-3	47.6
AB-4	50.7
AB-5	70.0
AB-6	53.4
AB-7	60.4
aminoguanidine	8.0

Best Mode for Carrying out the Invention

Examples:

The following are examples of pharmaceutical compositions of Maillard's reaction inhibitors of the present invention. Each code in the formulae represents each of the compounds described in the section of Pharmacological test.

(Example 1) Oral tablets

According to the formula below, the ingredients are formed into a tablet by a conventional method. Sugar coating may optionally be made.

AB-1	100 mg
lactose	80 mg
corn starch	17 mg
magnesium stearate	3 mg

(Example 2) Oral tablets

According to the formula below, the ingredients are formed into a tablet by a conventional method. Sugar coating may optionally be made.

AB-2	50 mg
corn starch	90 mg
lactose	30 mg
hydroxypropylcellulose	25 mg
magnesium stearate	5 mg

(Example 3) Capsules

According to the formula below, the ingredients are admixed and granulated by a conventional method and filled in capsules in an amount of 100 mg/capsule.

AB-3	10 mg
corn starch	45 mg
lactose	20 mg
crystalline cellulose	24 mg
talc	0.5 mg
magnesium stearate	0.5 mg

(Example 4) Injection

According to the formula below, the ingredients are admixed by a conventional method to dissolve. The solution is filtered, filled into vials and autoclaved to sterilize.

AB-4	20 mg
chlorobutanol	5 mg
water for injection	1 ml

(Example 5) Eye drops

According to the formula below, the ingredients are admixed by a conventional method to dissolve, and the solution is sterilized by filtration.

AB-5	0.5 g
boric acid	1.0 g
borax	q.s.(pH 7.0)
sodium chloride	0.25 g
disodium edetate	0.02 g
chlorobutanol	0.2 g
polysorbate 80	0.2 g
sodium sulfite	0.2 g
sterile purified water	to 100 ml

(Example 6) Eye ointment

According to the formula below, the ingredients are admixed by a conventional method to form an eye ointment.

AB-7	0.5 g
white vaseline	100 g

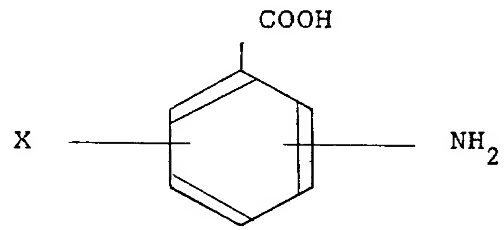
Industrial Applicability

The inhibitors of Maillard's reaction represented by the formula (I) and pharmaceutically acceptable salts thereof, inhibit the very formation of Amadori rearrangement product, the immediate causing factor of cross linkings among protein molecules.

The pharmaceutical compositions of the present invention, accordingly, may be useful for treatment and prophylaxis of diabetic complications, for example coronary heart disease, peripheral circulation disorders, cerebrovascular disorders, neuropathy, nephropathy, arteriosclerosis, arthrosclerosis, cataract and retinopathy, and age-associated disorders such as atherosclerosis, coronary heart disease, cerebrovascular disorders and senile cataract.

Claims

1. A Maillard's reaction inhibitor composition characterized in that it contains a compound represented by the formula (I),



(I)

wherein X denotes a hydroxyl group or a nitro group, a pharmaceutically acceptable ester thereof or a pharmaceutically acceptable salt of the said compound or the said ester.

2. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 5-hydroxyanthranilic acid.

3. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 3-hydroxyanthranilic acid.

4. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 4-nitroanthranilic acid.

5. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 5-aminosalicylic acid.

6. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 4-aminosalicylic acid.

7. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 3-aminosalicylic acid.

8. The Maillard's reaction inhibitor composition of Claim 1 wherein the compound represented by the formula (I) is 3-amino-4-hydroxybenzoic acid.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP91/00200

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl^5 A61K31/195

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61K31/195

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 56-7747 (May & Baker Ltd.), January 27, 1981 (27. 01. 81), (Family: none)	1-8

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
May 13, 1991 (13. 05. 91)	May 20, 1991 (20. 05. 91)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	